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THE MODIFICATION OF POLYMER SURFACES AND THE FABRICATION OF SUBMICRON-SCALE FUNCTIONALIZED STRUCTURES BY DEEP-UV AND ELECTRON BEAM LITHOGRAPHY.

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We present a general technique to modify polymer surfaces using N-hydroxysuccinimide (NHS) functionalized perfluorophenyl azides (PFPAs). Thin polystyrene films are spin-coated with a solution containing the NHS PFPA ester and are either UV photolyzed with a dosage of 10 mJ cm^{-2} or exposed with a 15 kV electron beam with a dosage between 1 and 75 μC cm^{-2}. The NHS active ester groups become covalently attached to the polymer via photogenerated or electron beam generated, highly reactive nitrene intermediates derived from the PFPA. Using this technique we demonstrate that well-defined surface regions can be functionalized with a minimum observable feature size of 0.5 μm and 0.2 μm for UV and electron-beam exposure, respectively. Through reaction of the functionalized surfaces with primary amine-containing reagents, we have installed biological molecules on the polymer and have measured the activity of an immobilized enzyme.

I. INTRODUCTION

Surface modification by the introduction of functional groups has been of recent interest for the development of resists,[1] biosensors,[2] and active devices. The combination of surface modification with microlithography offers precise control over the position of the surface-bound chemically reactive species that enables the immobilization of proteins,[3] cells[4] and other biomolecules[5] in a spatially defined fashion. Many surface modification techniques require a sequential chemical treatment of the surface.[6] Often this treatment is aggressive and involves complicated reaction conditions.

In contrast to the sequential chemical treatment of surfaces, a few studies have used azides as a surface modification agent.[7,8] Functionalized perfluorophenyl azides (PFPAs) were recently developed as photolabling agents.[9-12] Photochemical studies of PFPAs have shown that photolysis of PFPAs in hydrocarbon solvents efficiently produce CH insertion products via highly reactive nitrene intermediates.[13-18] Taking advantage of the efficient CH insertion property of the PFPAs, we have developed a general technique to modify polymer surfaces using N-hydroxysuccinimide (NHS) functionalized perfluorophenyl azides.[8]

Herein, we discuss the use of deep-UV lithography to functionalize well-defined patterns on a polymer surface by the photolysis of functionalized PFPAs. We also report the extension of the methodology to include the use of an electron beam as the reaction energy source for nitrene generation.

II. EXPERIMENTAL

The surface modification scheme was studied on thin films of polystyrene (PS). A silicon wafer was spin-coated with a solution of 5 wt % PS (MW 125,000 - 250,000) in xylene at 1000 rpm for 2 min to produce a PS film that was about 0.5 μm thick. A solution of 0.5 wt% of N-

hydroxysuccinimidyl 4-azido-2,3,5,6-tetrafluorobenzoate (NHS PFPA ester) in nitromethane was then spin-coated on top of the PS film at 1000 rpm for 1 min. The films were baked in an oven at 60 °C for 20 min. Two reaction energy sources were used to functionalized the surface: deep-UV and an electron beam. Deep-UV exposure was carried out at 254 nm with a KSM Karl Suss contact aligner using a high resolution photomask having a minimum feature size of 0.5 μm. The contact aligner had a UV intensity of 3.2 mW cm^{-2}. In another series of experiments, preformed PS microstructures were spin-coated with NHS PFPA ester, irradiated and developed. Electron beam exposure of the PS films was accomplished using a scanning electron microscope equipped with a pattern generator that was able to control the exposure dosage.[19] For both reaction energy sources, the exposed film was developed in nitromethane for 20 s then dried in a stream of dry air. After being developed, several different amine-containing reagents were immobilized on the functionalized patterns as described below and experiments were performed to study pattern integrity and enzymatic activity of the immobilized species. In all cases the results were compared with control experiments carried out on a nonfunctionalized PS surface. The decomposition of the azido groups was studied by comparing the FTIR spectrum of the films before and after photolysis. For this purpose PS films were prepared on a NaCl disk. The film was spin coated with a NHS PFPA ester and UV photolysis was carried out at 254 nm in a Rayonet reactor for 5 min.

III. RESULTS AND DISCUSSION

Under deep-UV photolysis the NHS PFPA ester produces a highly reactive nitrene intermediate that is very effective at CH insertion. Therefore, the most likely mechanism that mediates the covalent attachment of the NHS PFPA esters to the polymer surface is shown in scheme 1. This mechanism is supported by FTIR studies of the NHS PFPA ester coated PS films which showed that complete

decomposition of the azido groups had occurred at a UV dosage of about 10 mJ cm^{-2}.

Since NHS active esters readily react with a variety of primary amines to form amides, in principle, the NHS PFPA esters tethered to the surface offer a means to attach covalently a variety of primary amine-containing reagents to the polymer surface.

To study the immobilization of primary amine-containing reagents in well defined patterns, a fluorescent dye was immobilized on the functionalized PS film. The PS film was immersed in a solution of 5-(aminoacetamido)fluorescein in ethanol (4.0 mg/1.0 mL) at 25 °C for 1 hour. After immersion the film was soaked in ethanol overnight, rinsed with pure alcohol and dried in a stream of air to give **1** (scheme 1) containing attached fluorescein. The patterns were viewed with an optical microscope equipped with a fluorescein filter set (excitation wavelength 450-490 nm, emission wavelength > 510 nm). Typical patterns from the dye immobilized on the functionalized PS surface are shown in Fig. 1(a). The measured difference in the white light intensity between patterned and non-patterned regions of PS film **1** is 4:1. A control experiment without the NHS PFPA ester showed no visible patterns. The smallest features of the UV mask (0.5 μm) were resolved, but were slightly broadened probably due to diffraction effects.

Surface functionalization of PS films was also realized by electron beam irradiation. In this process an electron beam is scanned over the NHS PFPA ester coated PS surface to produce chemically reactive regions with written feature sizes down to 0.1 μm. Subsequent reaction with the fluorescein dye generated fluorescent patterns (scheme 1, film **1**) which could be studied under the fluorescence microscope. Accelerating voltages in the range 5 to 40 kV and dosages between 1 and 75 μC cm^{-2} were studied. For all dosages, the white light contrast of the patterns decreased above an accelerating voltage of 20 kV, while below 20 kV there was little discernable change in the patterns. Therefore, the effect of dosage was tested at an accelerating voltage of 15 kV. As shown in Fig. 1(b),

the higher the exposure the greater the white light contrast, however, above about 25 μC cm^{-2} the pattern resolution was found to degrade. The minimum written feature size that was observable by the technique was 0.2 μm which was broadened to about 0.5 μm, probably due to diffraction. A control experiment carried out on a nonfunctionalized PS surface showed no fluorescent pattern.

A comparison of Figs. 1(a) and 1(b), shows that the contrast obtained by electron beam functionalization is much weaker than that mediated by deep-UV. By analogy with electron-beam exposure of traditional positive and negative resists,[20] it seems likely that low-energy electrons back-scattered from the substrate decompose the azido group in the NHS PFPA ester and generate the nitrene intermediate which inserts into the CH bonds on the polymer surface (scheme 1). Therefore, the weaker contrast found in these nonoptimized experiments suggests that with a PS film thickness of 0.5 μm the efficiency of nitrene generation may be lower for energetic electrons than it is for the deep-UV photons. Alternatively, the high vacuum present in the electron microscope may have evaporated some of the NHS PFPA before beam exposure.

As a way to increase the contrast by reducing the residual fluorescence from the PS film, and obtain feature sizes below the diffraction limit of the exposing UV radiation, preformed PS ridge microstructures on a silicon substrate were UV functionalized. The PS microstructures were fabricated from a PS film, containing a bis(perfluorophenyl) azide cross-linking agent, by cross-linking with the electron beam.[21] The resulting ridge structures had a height of about 0.5 μm and widths in the range 0.1 to 10 μm. A solution of NHS PFPA ester in nitromethane was spin-coated over the ridge structures, deep-UV photolyzed and developed as indicated above. The fluorescein dye was then immobilized on the structures. It is seen from the fluorescence micrograph of the structures shown in Fig. 2 that the smallest feature is resolved. To determine whether there was non-specific absorption of the fluorescein dye on the PS ridges a control experiment performed without functionalization.

Again almost no fluorescence was discernable.

It is well known that biotin is bound strongly and specifically by the bacterial protein avidin with a binding constant of 10^{15} M^{-1}.[22] To study the possibility of patterning through the use of a biotin-avidin linkage, an avidin-fluorescein conjugate was immobilized on a PS-preformed microstructure by way of binding to an immobilized N-(5-aminopentyl)biotinamide. This latter compound is an amine-containing biotin derivative that retains high affinity toward avidin. The surface of the PS-preformed microstructure was functionalized with NHS PFPA ester as described above. The patterned film was then dipped in a solution of the amino-biotin in dimethylformamide (DMF) at 25 °C for 4 hours and washed by DMF followed by ethanol to give PS-biotin 2 (scheme 1). Thus, amino-biotin was covalently attached to the PS patterned surface through amide formation between the amino group in the biotin and the NHS group. The film was then immersed in a solution of avidin-fluorescein conjugate in a buffer for 4 hours, washed by the buffer, water, ethanol and air-dried to give PS-biotin-avidin-fluorescein 3. Figure 3 shows the patterns visualized under a fluorescence microscope. Two control experiments were performed. A nonfunctionalized PS-preformed microstructure surface was treated with amino-biotin followed by avidin-fluorescein conjugate. In another control experiment, a nonfuctionalized PS-preformed microstructure was treated directly with avidin-fluorescein conjugate. Both control samples showed only weak fluorescence under the fluorescence microscope indicating that there was little non-specific absorption of either amino-biotin or avidin-fluorescein conjugate on the PS film.

Similar biotin experiments and controls were performed on PS films using electron beam functionalization at 15 kV and dosages in the range 15 to 40 $\mu C\ cm^{-2}$. In this case, after reaction with the avidin-fluorescein conjugate, both the functionalized surface and nonfunctionalized control area free of NHS PFPA ester but exposed to the electron beam showed fluorescent patterns with a minimum

feature size of about 0.5 μm. In some cases the control samples had a greater white light contrast than the functionalized samples. One possible explanation for the observation of fluorescent patterns on the control sample is electrostatic attachment of the avidin-fluorescein in a pH 8.2 buffer to the PS by volume negative charge created by the electron beam. It is expected that the relaxation time of any trapped charge will be $\tau \approx \varepsilon\rho$, where ε is the dielectric constant and ρ is the resistivity of PS. Taking $\varepsilon = 2.2 \times 10^{-11}$ F m^{-1} and $\rho \geq 10^{17}$ Ω cm, we expect $\tau \geq 6$ hours, which is longer than the time take to perform the chemical reactions. To test the hypothesis of electrostatic attachment, PS was electron beam exposed and then reacted with polylysine in a pH 8.2 buffer and then reacted with avidin-fluorescein. Following this procedure no fluorescent patterns were observed: Indeed, the white light contrast of the pattern became negative demonstrating that the residual fluorescence of the PS was greater than that of the polylysine. This result showed that the multiply positively charged polylysine became attached to the charged regions of the PS, screened the trapped charge and thereby prevented the avidin-fluorescein from binding to the surface. Reaction with amino-biotin followed by avidin-fluorescein was also carried out on a control sample 24 hours after electron beam irradiation, that is many times the relaxation time τ As expected no fluorescent pattern was observed. Further details of binding molecules to patterned charged regions will be discussed elsewhere.[23]

Finally, the enzyme horseradish peroxidase (HRP) was immobilized on PS films.[8] The enzymatic activity was used to determine the immobilization efficiency. Non-patterned NHS functionalized PS films were incubated in a 50 μM solution of HRP in a buffer for 3 hours at 25°C,[24] to give PS-HRP 4. The enzymatic activity was determined spectrophotometrically at 420 nm.[25] Provided that the immobilized HRP has the same activity as native HRP, the extent of the immobilization was 0.5 ± 0.1 ng mm^{-2}. A HRP molecule has a molecular weight of about 40,000 and a radius of 2.67 nm in the hydrated state.[26] Assuming a flat polymer surface, a maximum surface

coverage of about 2.7 ng mm^{-2} is expected for a monolayer of HRP. Therefore, our data suggest a coverage of about 20% indicating a reasonable immobilization efficiency in these nonoptimized experiments. More details of the HRP immobilization will be reported elsewhere, together with the effects of introducing spacers between the NHS PFPA ester and the HRP.[27]

IV. CONCLUSION

We have demonstrated that the combination of the surface modification using N-hydroxysuccinimide (NHS) functionalized perfluorophenyl azide with deep-UV or electron beam lithography can be used to form closely-defined regions of functionalized polymer surface. The advantage of this surface functionalization process is that it is versatile, yet relatively straightforward, and can produce patterns with a feature size as small as 0.2 μm. We have also shown that an enzyme can be installed on a polymer surface and that enzymatic activity is retained. This surface functionalization technique should be of general use for the delineation of a variety of biomolecular microstructures and may find applications in constructing novel micro-biosensors and nucleotide screening assays.

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FIGURE CAPTIONS

Figure 1 Micron-size features taken under a fluorescence microscope showing the surface modification of a PS film (a) by deep-UV lithography; (b) by electron beam lithography. The drawn linewidths are 5, 2, 1, 0.5, 0.2, and 0.1 μm in each group. Counter-clockwise from the upper left hand group, the dosages are 15, 25, 35, and 45 $\mu C\ cm^{-2}$.

Figure 2 Micrograph taken under a fluorescence microscope with a fluorescein filter set showing the PS-preformed microstructures after treatment with NHS PFPA ester followed by 5-(aminoacetamido)fluorescein.

Figure 3 Micrograph taken under a fluorescence microscope with a fluorescein filter set showing the surface modification of PS-preformed microstructures after treatment with NHS PFPA ester followed by N-(5-aminopentyl)biotinamide, then avidin-fluorescein conjugate.

REFERENCES

1. MacDonald, S. A.; Schlosser, H.; Ito, H.; Clecak, N. J.; Willson, C. G. *Chem. Mater.* 1991, 3, 435-442.

2. Pantano, P.; Morton, T. H.; Kuhr, W. G. *J. Am. Chem. Soc.* 1991, 113, 1832-1833.

3. Bhatia, S. K.; Hickman, J. J.; Ligler, F. S. *J. Am. Chem. Soc.* 1992, 114, 4432-4433.

4. Stenger, D. A.; Georger, J. H.; Dulcey, C. S.; Hickman, J. J.; Rudilph, A. S.; Nielsen, T. B.; McCort, S. M.; Calvert, J. M. *J. Am. Chem. Soc.* 1992, 114, 8435.

5. Rozsnyai, L. F.; Benson, D. R.; Fodor, S. P. A.; Schultz, P. G. *Angew. Chem. Int. Ed. Eng.* 1992, 31, 759-761.

6. Fodor, S. P. A.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science* 1991, 251, 767-773.

7. Harmer, M.A. *Langmuir* 1991, 7, 2010-2012.

8. Yan, M.; Cai, S. X.; Wybourne, M. N.; Keana, J. F. W. *J. Am. Chem. Soc.* 1993, 115, 814.

9. Cai, S. X.; Keana, J. F. W. *Bioconjugate Chem.* 1991, 2, 38.

10. Pinney, K. G.; Katzenellenbogen, J. A. *J. Org. Chem.* 1991, 56, 3125.

11. Crocker, P. J.; Rajagopalan, K.; Boggess, M. A.; Kwiatkowski, S.; Dwyer, L. D.; Vanaman, T. C.; Watt, D. S. *Bioconjugate Chem.* 1990, 1, 419.

12. Aggeler, R.; Chicas-Cruz, K.; Cai, S. X.; Keana, J. F. W.; Capaldi, R. A. *Bioconjugate Chem.* 1992, 31, 2956.

13. Keana, J. F. W.; Cai, S. W. *J. Org. Chem.* 1990, 55, 3640.

14. Keana, J. F. W.; Cai, S. W. *J. Fluorine Chem.* 1989, 43, 151.

15. Cai, S. X.; Keana, J. F. W. *Tetrahedron Lett.* 1989, 30, 5409.

16. Schnapp, K.; Poe, R.; Leyva, E.; Soundararajan, N.; Platz, M. S. *Bioconjugate Chem.* 1993,

4, 172.

17. Poe, R.; Schnapp, K.; Young, M. J. T.; Grayzar, J.; Platz, M. S. *J. Am. Chem. Soc.* 1992, 114, 5054.

18. Soundararajan, N.; Platz, M. S. *J. Org. Chem.* 1990, 55, 2034.

19. Nabity, J. C.; Wybourne, M. N. *Rev. Sci. Instrum.* 1989, 60, 27.

20. Moreau, W.M.; Semiconductor Lithography Principles, Practices, and Materials; Plenum Press: New York, 1988.

21. Cai, S. X.; Nabity, J. C.; Wybourne, M. N.; Keana, J. F. *Chem. Mater.* 1990, 2, 631.

22. Bayer, E. A.; Wilchek, M. In *Methods of Biochemical Analysis* Glick, D. Ed.; John Wiley & Sons: New York, 1980, Vol 26, p 1-46.

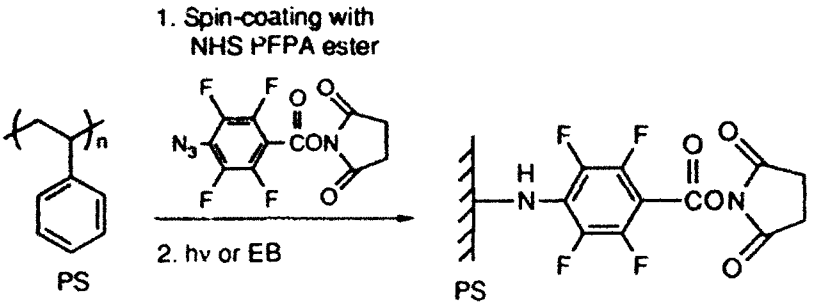
23. Yan, M.; Wu, J.C.; Keana, J.F.W.; Wybourne, M.N.; To be published.

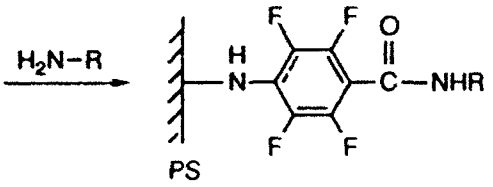
24. Brinkley, M. *Bioconjugate Chem.* 1992, 3, 2.

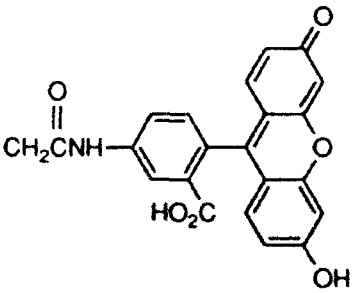
25. Groome, N. P. *J. Clin. Chem. Clin. Biochem.* 1980, 18, 345.

26. Steiner, H.; Dunford, H.B.; *Eur. J. Biochem.* 1978, 82, 543-549.

27. Yan, M.; Cai, S.X.; Wybourne, M.N.; Keana, J.F.W.; To be published.



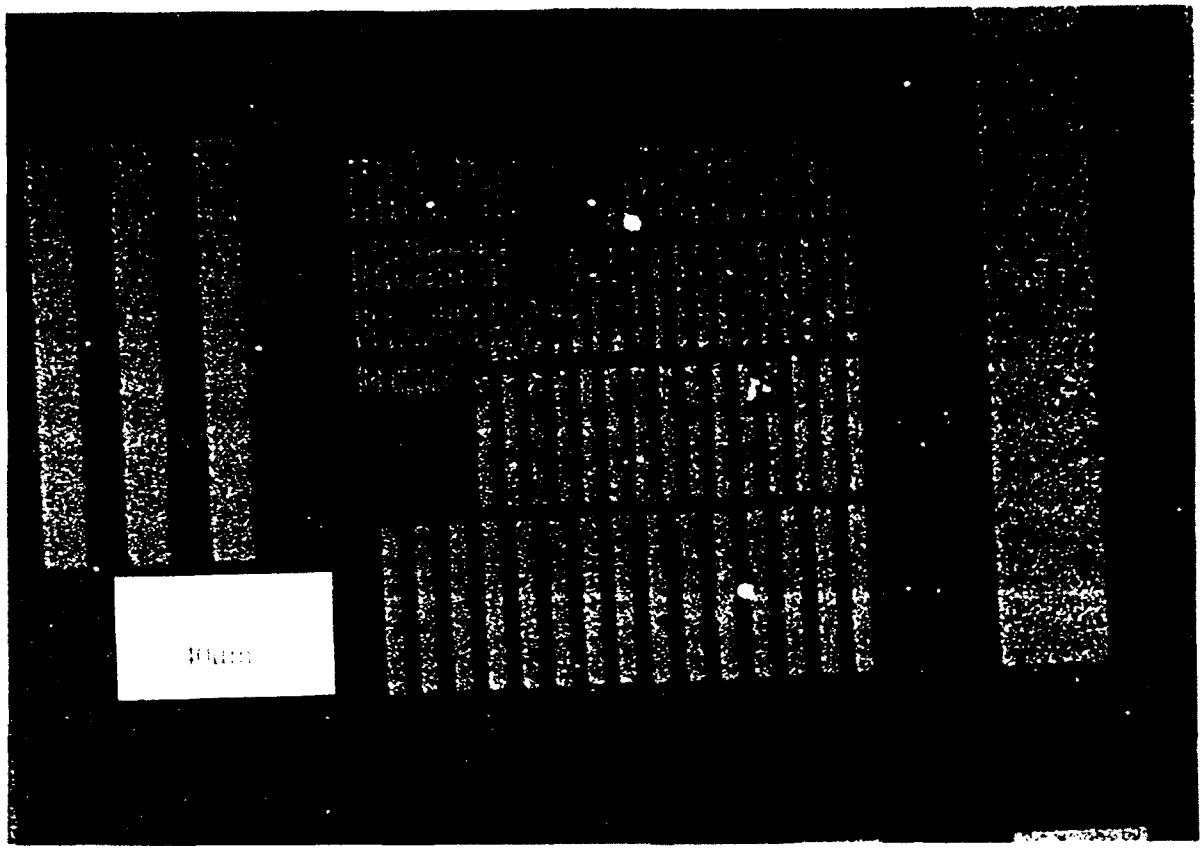


1 R =  (attached fluorescein)

2 R = $(CH_2)_5NC$–biotin (attached biotin)

avidin-fluorescein → **3** R = $(CH_2)_5NC$–biotin-avidin-fluorescein

4 R = horseradish peroxidase (attached horseradish peroxidase)



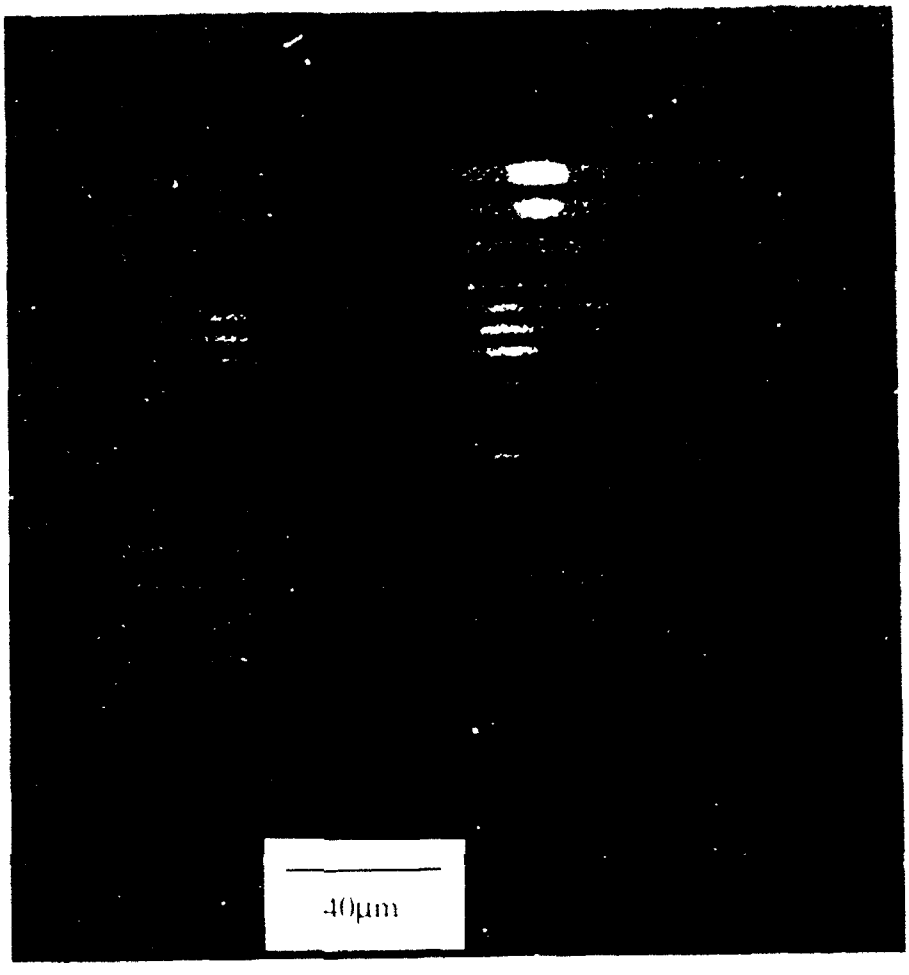
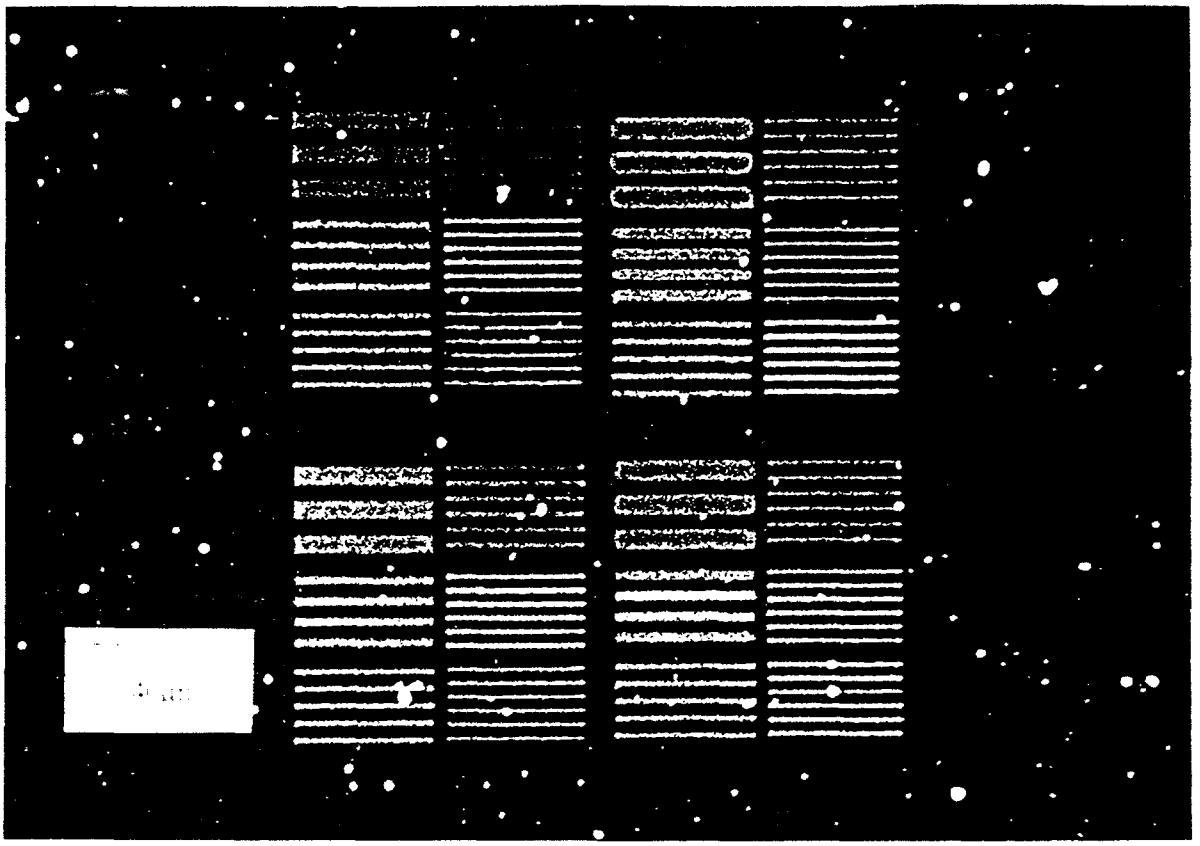


Fig 4(b)



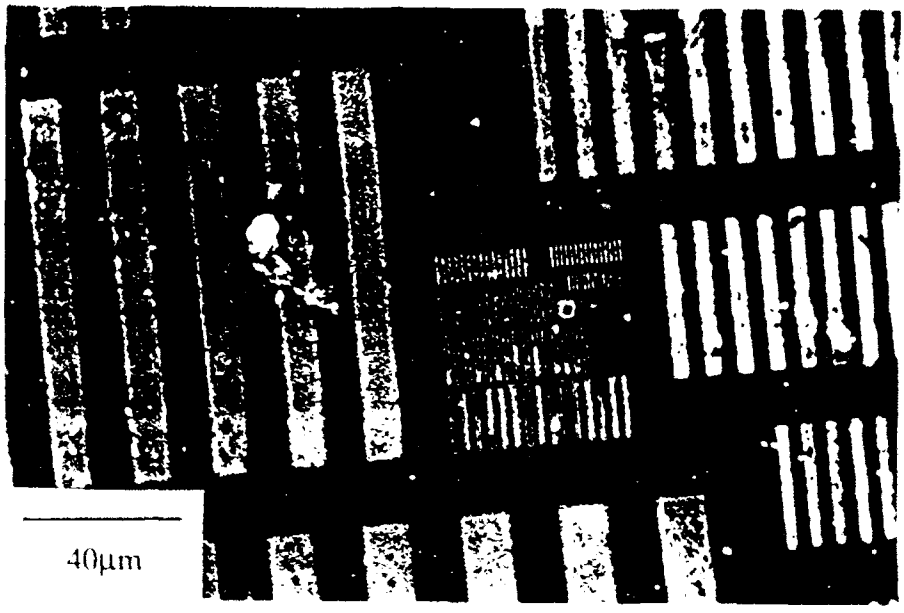


Fig. 2

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